



**ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF BACTERIA ISOLATED FROM
STERILE SITES: CEREBRAL SPINAL FLUID, BLOOD, PERITONEAL
FLUID, PLEURAL FLUID AND SYNOVIAL FLUID AT
KENYATTA NATIONAL HOSPITAL.**

A Project submitted in Partial Fulfillment for the Award of Master of Science Degree in Tropical and Infectious Diseases from University of Nairobi, Institute of Tropical and Infectious Diseases UNITID.

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DEDICATION

To my family which encouraged me and gave tremendous support through the duration of this project.

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ABBREVIATIONS

AFB- Acid Fast Bacilli

AIDS- Acquired Immunodeficiency Syndrome

AMR- Antimicrobial Resistance

AST- Antimicrobial Susceptibility Testing

BA- Blood Agar

CBA-Chocolate Blood Agar

CNS-Central Nervous System

CSF- Cerebrospinal Fluid

ERC- Ethics Review Committee

ESKAPE (*Enterobacter, S.aureus, K.Pneumonia, A.baumannii, P.aeruginosa, E.faecium*)

HIV- Human Immunodeficiency Virus

KNH- Kenyatta National Hospital

NNISS - National Nosocomial Infections Surveillance System

UON- University of Nairobi

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ABSTRACT

Background. Antimicrobial resistance is dramatically increasing worldwide. Much of it due to inappropriate overuse and is causing significant morbidity and mortality.

Diagnosis of sterile site infections is based on culture of properly collected and processed samples. Since definitive diagnosis is based on quantitative cultures, the course of antibiotic therapy should be determined after the culture results have been confirmed. Unfortunately in most instances empiric treatment is commenced because it is not possible to wait for culture reports or laboratory facilities are unavailable.

Infections caused by drug resistant organisms are difficult to eradicate because of limited therapeutic options. With growing antimicrobial resistance in Kenya, reliance on international guidelines is insufficient and hence a study such as this one is needed to get our local patterns to help formulate local policies.

Objectives. The general objective of this study was to determine the bacterial isolates identified from sterile body sites and their antibiotic susceptibility patterns from both inpatients and outpatients at the Kenyatta National Hospital (KNH) microbiology laboratory, in the period January to December 2013.

Study design and Methodology. This was a retrospective descriptive study done over three months using previously available data from the patients' laboratory files.

After obtaining ethical approval from the KNH/UON- ERC, abstraction of data of samples collected from sterile sites was done from the existing laboratory database using a coded form, which was then recorded on a tally sheet. The outcomes that were considered were bacterial isolates from the respective sterile sites i.e. Cerebral Spinal Fluid (CSF), blood, peritoneal, pleural and synovial fluid; and their antibiotic susceptibility patterns. Demographic

characteristics such as age and sex were also looked at. Data was then analyzed using Statistical Package for Social Sciences Programme (SPSS) version 17.0 by univariate and bivariate analysis.

Results. A total of 63 organisms identified from the various sites and included *Staphylococci*-21 (33.3%), *Enterobacteriaceae*-28 (44.4%), *Enterococci*-7 (11%), *Pseudomonas*-2 (3.2%), *Aer. sobria*-2 (3.2%) and one isolate each of *Aci. baumannii*, *Strep. agalactiae* and *Pantoea* (1.6%).

Among *Staphylococci*, 81% were sensitive to vancomycin which was the only drug that was tested in VITEK 2. *Enterobacteriaceae* showed sensitivity against piperacillin/tazobactam, cefoxitin, cefepime, amikacin and meropenem, and resistance against ampicillin and cefuroxime. *Pseudomonas* isolates both showed sensitivity towards ceftazidime and amikacin with resistance to ampicillin, piperacillin/tazobactam, cefuroxime, cefoxitin. *St. agalactiae* had sensitivity towards both ampicillin and vancomycin. *Aer. sobria* was sensitive towards cefepime and amikacin. *Pantoea* showed sensitivity towards cefepime and meropenem and resistance against ceftazidime and piperacillin/tazobactam. *Aci.baumannii* was resistant to all drugs tested against it: ampicillin, piperacillin/tazobactam, ceftazidime, cefuroxime and cefoxitin.

Conclusion and Recommendations. Emerging ESKAPE (*Enterobacter*, *S.aureus*, *K.Pneumonia*, *A.baumannii*, *P.aeruginosa*, *E.faecium*) organisms have been isolated in this study and remain important pathogens as far as infections in sterile sites are concerned. Commonest organisms from blood were *Staphylococci* and *Klebsiella*, and from ascitic fluid were *Enterobacteriaceae*.

Surveillance especially for the emerging pathogens needs to be carried out judiciously to help develop rational antimicrobial guidelines alongside continuous medical education. Rational use of antibiotics is advised to help curb this trend of increasing antibiotic resistance.

CHAPTER 1

1.0 BACKGROUND

An antimicrobial is an agent that kills microorganisms such as bacteria, viruses or fungi, or suppresses their multiplication or growth. Antibiotic susceptibility is the inhibition of growth or killing of bacteria by use of antibiotics.

Acquisition of Antimicrobial resistance (AMR) is resistance of a microorganism to an antimicrobial agent to which it was originally sensitive. Resistant organisms are able to withstand attack by antimicrobial medicines, such as antibiotics, antifungals and antivirals such that standard treatments become ineffective and infections persist.

Resistant factors can be exchanged between certain types of bacteria when microorganisms are exposed to antimicrobial drugs, causing them to evolve naturally into resistant strains.

The resistance rate to antibiotics has been on the increase due to factors such as inappropriate use of antimicrobial medicines; either misuse or overuse such as treating viral infections with antibiotics and poor infection prevention and control practices. On the contrary, underuse of antibiotics also contributes to resistance through inadequate dosing and poor compliance by patients hence according the micro-organisms opportunities to multiply and continue spreading (WHO, 2011).

Additionally, weak or absent antimicrobial resistance surveillance systems makes it difficult to acquire the necessary data needed to assess and improve antibiotic use.

According to Centre for Disease Control Morbidity and Mortality Weekly Report (2013), data emerging from different parts of the world have suggested that strains of highly multidrug

resistant organisms have quadrupled in the past decade. This emergence can not be ignored as the risk of morbidity and mortality is heightened when they afflict vulnerable individuals.

As AMR is a complex multifaceted problem, single isolated interventions have little impact in curbing it and coordinated actions between different stakeholders starting from the patient to the healthcare providers and national governments are required to effectively tackle it.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 STERILE BODY SITES, PATHOGENS AND CONTAMINANTS

Sterile body sites are those in which no bacteria or microbes exist as commensals when in a healthy state. This can either be pathological agents or contaminants from skin or laboratory processes and include blood, CSF, peritoneal fluid, pleural fluid and synovial fluid. Non-sterile samples are those obtained from sites considered not sterile and there may be colonizing microbial agents. The significance of the isolates obtained is through the density of growth, for example in urine $>10^5$ colony-forming units (CFU) of bacteria per milliliter of urine, formed colonies and in skin Bacillus growth versus *S.epidermidis*.

2.1.1 Cerebral Spinal Fluid:

CSF is a clear colorless bodily fluid found in the brain and spine whose primary function is to cushion the brain within the skull and serve as a shock absorber for the central nervous system. CSF also circulates nutrients and chemicals filtered from the blood and removes waste products from the brain. It occupies the subarachnoid space (the space between the arachnoid and the pia mater) and the ventricular system around and inside the brain and spinal cord (Wikipedia).

Various studies from India revealed that meningitis is caused by various pathogens depending on the patient's age group. In neonates, Group B (49%) and non-Group B *Streptococcus* species, *Escherichia coli* (18%), and *Listeria monocytogenes* (7%) are the most common causative

organisms. Children and infants acquire meningitis from infection with *Haemophilus influenzae* (40-60%), *Neisseria meningitidis* (25-40%), and *Streptococcus pneumoniae* (10-20%). The sources of adult meningitis include *S. pneumoniae* (30-50%), *N. meningitidis* (10-35%), *Staphylococcus* (5-15%), other *Streptococcus* species, *H. influenzae* (1-3%), Gram-negative bacilli (1-10%), and *L. monocytogenes* (Chandramukhi, 1989; Chinchankar, 2002; Sonavane, 2008).

Unlike the community acquired bacterial meningitis, gram-negative bacilli (40-60%) and staphylococci, mainly coagulase negative (30-50%), are the most common causative agents of nosocomial meningitis (Krcmery,2000).

Latex Agglutination Test is an adjunct to conventional techniques in the diagnosis of pyogenic bacterial meningitis, where the latter tests fail. It is used for detection of the antigens of *Streptococcus pneumoniae*, *Group B Streptococci*, *Escherichia coli*, *Neisseria meningitidis* and *Haemophilus influenzae* type b. It was originally designed to be used in patients who demonstrated laboratory and clinical findings consistent with meningitis. However it has been used much too often as screening tool in cases of suspected meningitis in patients whose CSF specimens have normal chemistries and cell counts (Kiska, 1995).

2.1.2 Blood

Blood culture is required when bacteraemia (the presence of bacteria in the blood) or septicaemia is suspected. It usually occurs when pathogens enter the bloodstream from abscesses, infected wounds or burns, or from areas of localized disease as in pneumococcal pneumonia, meningitis, pyelonephritis, osteomyelitis amongst others.

Septicaemia occurs when multiplying bacteria release toxins into the blood stream and trigger the production of cytokines, causing fever, chills, toxicity, tissue anoxia, reduced blood pressure and collapse and can complicate as septic shock.

Bloodstream infections cause significant morbidity and mortality worldwide and are among the most common healthcare-associated infections, with mortality rates approaching 45% in bacteremia due to gram negative organisms (Blot, 2002).

Diekema (2003) compared community-onset and nosocomial bloodstream infections and found that Gram-positive pathogens caused the majority of both with *Staphylococcus aureus* being the most common pathogen overall. Specifically, *Escherichia coli* was the most common cause of community-onset bloodstream infection, whereas *S. aureus* caused similar proportions of both community-onset (18%) and nosocomial (21%) bloodstream infections.

A study done by Deverick (2014) showed that healthcare exposure preceded the onset of blood stream infections in almost 3 of every 4 patients in their cohort, as evidenced by the fact that majority of the patients in that cohort had central venous lines and had invasive devices present at the time of infection.

However, findings by Weinstein (1997) showed that only 50% of all positive blood cultures represent true bloodstream infection.

Blood Contaminants:

A significant proportion of cases have been found to be contaminated with certain organisms which include Coagulase Negative Staphylococci (most common), *Corynebacterium* species, *Bacillus* species other than *Bacillus anthracis*, *Propionibacterium acnes*, *Micrococcus* species, *Viridians* group streptococci, enterococci, and *Clostridium perfringens* (Weinstein, 2003,1997) .

According to National Nosocomial Infections Surveillance (NNIS) System (1991), coagulase-negative bacteremia is often the result of long-term use of indwelling central and peripheral catheters as well as other prosthetic devices, the ubiquity of these bacteria as normal skin flora, and the ability of these relatively avirulent organisms to adhere to the surface of biomaterials .

With particular regard to Coagulase Negative Staphylococci, growth of 2-5% is considered as contamination; >5% shows poor infection control or swabbing practices and <2% shows a high risk of laboratory overprocessing. However, it is crucial to recognize that each of these organisms can also represent true bacteremias with devastating consequences, particularly if untreated due to misinterpretation as contaminants.

BODY CAVITIES

An effusion is fluid which collects in a body cavity or joint. Fluid which collects due to an inflammatory process such as infections or malignancy is referred to as an exudate and that which forms due to a non-inflammatory condition is referred to as a transudate.

2.1.3 Peritoneal Fluid

Ascitic (peritoneal) fluid is from the peritoneal (abdominal) cavity. Peritonitis means inflammation of the peritoneum, which is the serous membrane that lines the peritoneal cavity. It can be caused by the rupture of an abdominal organ, or as a complication of bacteraemia or can be spontaneous. Peritoneal dialysis is also associated with a high risk of infection of the peritoneum, subcutaneous tunnel and catheter exit site.

The causative organisms in peritoneal dialysis peritonitis are generally different to those causing surgical peritonitis. In surgical cases, infections are usually poly-microbial consisting of both anaerobic and aerobic bacteria. In contrast, a single micro-organism, usually a skin-colonising Gram-positive bacteria, is the common cause of peritoneal dialysis peritonitis; *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus spp.* account for 60-80% of cases. (Brook, 2004)

However, it must be kept in mind that a significant proportion of the infections are culture negative - about 20% to 32.5% (Lobo, 2010) and so appropriate samples should be obtained prior to commencing treatment. It is recommended that evaluation of culture-negative Peritoneal dialysis-related infections be done for rapidly growing nontuberculous mycobacteria infections especially in the clinical setting of non-resolving peritonitis after prior exposure to antibiotics (Renaud, 2011).

Unfortunately, the prolonged turnaround time of 1 to 2 days of culture limits its utility for directing antibiotic selection in acute care settings.

2.1.4 Pleural Fluid

This is fluid from the pleural cavity i.e. space between the lungs and the inner chest wall. Pleural effusion is used to describe a nonpurulent serous effusion which sometimes forms in pneumonia, tuberculosis, malignant disease, or pulmonary infarction (embolism), Systemic Lupus Erythematosus, lymphoma, rheumatoid disease, or amoebic liver abscess.

Common bacterial pathogens include *Streptococcus milleri* group species, *Streptococcus pneumoniae*, Methicillin sensitive *Staphylococcus aureus* (MSSA) and the *Enterobacteriaceae* group (Foster, 2007; Meyer, 2011)

S. aureus is more commonly seen in the older, hospitalized patient with co-morbidities and is associated with cavitation and abscess formation, with empyema present in 1-25% of adult cases.

(Lindstrom,1999). Anaerobic bacteria however contribute significantly to pleural infection, being identified as the sole or co-pathogen in 25-76% of pediatric cases (Micek, 2005).

The causative microorganism is, however, only identified in approximately 50% of cases.

Empyema is used to describe a purulent pleural effusion when pus is found in the pleural space.

It can occur with pneumonia, tuberculosis, infection of a haemothorax (blood in the pleural cavity), or rupture of an abscess through the diaphragm. Common organisms associated with

empyema include: *Staphylococcus aureus*, *Haemophilus influenza*, *Streptococcus pneumonia*, *Bacteroides*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Actinomycetes*, *Klebsiella strains*, *Mycobacterium tuberculosis*. Organisms such as Methicillin Resistant *Staphylococcus aureus*, *Enterobacteriae* and anaerobes are more prevalent in nosocomial empyema (Schultz,

2004)

Worldwide, *Mycobacterium tuberculosis* is one of the most important causes of pleural infection.

In immunocompetent patients Acid Fast Bacilli smear in pleural effusion is rarely positive and in HIV patients it's positive in 20%. Pleural fluid culture is positive approximately 40% of patients. In order to achieve a definitive diagnosis of tuberculous pleurisy, *Mycobacterium tuberculosis* must be isolated from the culture of pleural fluid or tissue; the presence of granulomas in pleural tissue is suggestive. (Valdes, 2003)

Choice of antibiotic should be informed by the results of blood and pleural fluid cultures and sensitivities; empirical anaerobic antibiotic cover should be considered as anaerobes frequently coexist but are difficult to isolate.

2.1.5 Synovial Fluid

Synovial fluid is the thick colourless lubricating fluid that surrounds a joint and fills a tendon sheath. It is secreted by the synovial membrane which lines the joint capsule, and when inflamed this is known as synovitis. Causes can be due to bacteria, rheumatic disorder, or injury. Infective synovitis is usually secondary to bacteraemia.

Organisms involved in both synovitis and arthritis include *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *Streptococcus pyogenes*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, Anaerobic streptococci *Brucella* species, Actinomycetes, *Salmonella* serovars, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus*, *Bacteroides* and *Mycobacterium tuberculosis*.

While *S. aureus*, group B streptococci and Gram-negative organisms are isolated in newborn infants, in older infants *Haemophilus influenzae* becomes a prominent pathogen (Sequeira, 1985). In those over 2 years of age, staphylococci, streptococci, *H. influenzae* and *Neisseria gonorrhoeae* are predominant organisms (Barton, 1987). *S. aureus* was found to be the commonest cause of septic arthritis in children by Wang (2003), however in other studies it was not confined to any age group (Barton, 1987; Welton, 1986). The gold-standard test for diagnosis of septic arthritis is synovial fluid culture which is positive in 80 % of cases according to Wang (2003).

2.2 CLINICAL IMPORTANCE AND IMPLICATIONS OF CURRENT PRACTICES OF ANTIMICROBIAL USE.

There is dramatic increase of antimicrobial resistance worldwide in response to antibiotic use, and is causing significant morbidity and mortality. It has been estimated that antimicrobial

resistance costs the health-care system in excess of US\$ 20 billion in the USA annually and generates more than 8million additional hospital days (Roberts, 2009).

A recent situation analysis by Kariuki (2011) in Kenya showed that three main factors contribute greatly to Antimicrobial resistance which include:

1. Burden of Infectious disease :

The top five killers in Kenya are Infectious diseases with Acute respiratory infections being the second leading cause with pneumonia as the largest contributor to the burden of disease among children living in urban informal settings, followed by diarrhoeal diseases.

2. Healthcare Environment and Behaviour

Antibiotics are also misused, their effectiveness wasted in patients with conditions that cannot be cured by antibiotics. Possible reasons for this include: lack of microbiology facilities and diagnostic capacity; fear of negative outcomes if antibiotics are withheld, particularly with malaria patients and limited access to formal healthcare services and the prevalence of self-medication.

3. Antibiotic Use in Animals

Evidence on antibiotic use in farm animals indicates that these medicines are used primarily (90%) for therapeutic applications. There is no regulation of antibiotic use in Kenya and no surveillance is done for effectiveness of these drugs. This will greatly impact on the susceptibility of most pathogens even those causing human disease. It has been postulated that not only clonal spread of resistant strains occurs, but also transfer of resistance genes occurs between human and animal bacteria.

Ecological factors:

Antimicrobial resistant bacteria, like antibiotic-susceptible bacteria can spread, from person to person to the environment, and then back to humans. In addition, the genes that encode antimicrobial resistance are often readily transferable from resistant to susceptible microorganisms, which can then multiply, spread and act as a source of further transfer of resistance genes. Infection prevention and control activities such as proper hygiene and sewage disposal to limit the spread of resistant bacteria are therefore crucial.

2.3 JUSTIFICATION

Sterile sites are those in which no bacteria or microbes exist as commensals when in a healthy state, such that any growth is considered significant and can either be pathogenic microorganisms or contaminants. On the contrary, for non-sterile sites, not all isolated organisms are significant as they can be normal flora.

Diagnosis of sterile site infections is based on culture of properly collected and processed samples. Preliminary reports such as gram staining, biochemistry and cytology may be helpful in providing immediate information to support the diagnosis and justify initiation of antibiotic treatment. However since definitive diagnosis is based on quantitative cultures, the course of antibiotic therapy should be determined after the culture results have been confirmed.

Unfortunately in most instances it is either not possible to wait for culture reports hence empiric treatment is commenced, or laboratory facilities are unavailable or unreliable.

Infections caused by drug resistant organisms are difficult to treat hence limiting the therapeutic options for treatment. With growing antimicrobial resistance in Kenya, reliance on international guidelines is insufficient. There is need of research such as this one to get antibiotic susceptibility testing profiles for pathogens from sterile site infections in our local setting, to enable us formulate local guidelines for treatment.

2.4 RESEARCH QUESTION

What is the prevalence and antibiotic susceptibility patterns of bacterial isolates from sterile body sites from both inpatients and outpatients at the KNH microbiology laboratory in the period January to December 2013?

2.5 OBJECTIVES

2.5.1 Broad Objective

To describe the bacterial isolates identified from sterile body sites and their antibiotic susceptibility patterns from both inpatients and outpatients at the KNH microbiology laboratory, in the period January to December 2013.

2.5.2 Specific Objectives

1. To describe bacterial isolates identified in cultures and their distribution in body fluids collected from sterile sites: CSF, blood, pleural, ascitic and synovial fluids.
2. To describe the antibiotic susceptibility patterns of isolated bacteria to various antibiotic agents.

CHAPTER 3

3.0 STUDY DESIGN AND METHODOLOGY

3.1 Study design

This was a retrospective descriptive study using previously available data from the KNH patients' laboratory files.

3.2 Study area

The study was based in Kenyatta National Hospital Medical Microbiology Laboratory. KNH is located in the Kenyan capital city of Nairobi. It is the largest teaching and referral for East and Central Africa and provides services to patients within the catchment area. It serves as one of the only two tertiary referral centers in the country and is also a training institute for doctors housing the University of Nairobi, College of Health Sciences.

3.3 Study Population

Data was abstracted from the KNH Microbiology laboratory records of both inpatients and outpatients of samples collected from sterile sites.

Inclusion criteria:

Laboratory records of all patients from whom bacterial isolates were cultured from sterile body sites during the period January to December 2013.

Exclusion Criteria:

Laboratory records of samples with no growth obtained.

3.4 Sampling

There was no sampling the number of sterile site samples in the period January to December 2013. The whole population was included in the study. The whole population was eligible for the study. Initially 74 samples were thought to be eligible but 11 ended up being contaminants bringing the population size to 63.

This was done in the Microbiology Laboratory archives, where materials were archived for each sample collected. Information on sterile sample sites specimen was picked; then each was run through manually to check the ones positive for growth. Thereafter sequential data extraction of isolates from sterile body sites was done from January to December 2013, through file reports and registers and also summary of Vitek 2 backup data which is a computerized software that analyses and stores data for epidemiological review (Appendix 5.4.3).

3.5 Data collection, entry and validation

Abstraction of data using a coded form was done from the existing laboratory database which was recorded on a tally sheet for the time period January to December 2013. All the data collection tools were reviewed by the principle investigator to ensure proper data management was done. Data collected was entered into a database in a password protected computer and a backup.

3.6 Procedures

The procedures that were used in the study were Data Collection form and Laboratory Procedures.

3.6.1 Data Collection Form

This is a tool that was used to gather information on variables of interest, in a systematic fashion that would enable us to answer the stated research questions, test hypotheses, and evaluate outcomes. It was adapted from a previous study done by Dr Simon Njiru in Institute of Tropical and Infectious Diseases (Appendix 5.4.1).

3.6.2 Laboratory Procedures

The procedures that were used in the laboratory to isolate and identify isolates and the antimicrobial susceptibility patterns during the period January to December 2013 have been elaborated further in Appendix 5.4.2.

3.7 Ethical Issues

Ethical approval prior to conducting the research was obtained from KNH/UON Ethics and Research Committee. Permission to extract data from the hospital registers and records was obtained from the KNH Head of Laboratory Medicine through KNH Research Office.

The study was a minimal risk study as there was no direct patient involvement but a retrospective review of the records.

For confidentiality, the patients' files were used within the confines of the KNH microbiology Laboratory and only the investigator with the assistance of research assistants and laboratory personnel would access the files for purposes of this study.

Patient identifying information such as the name and patient numbers were not included in the data collection forms. The report registers when not in use were kept under lock and key in the Microbiology Laboratory archives.

3.8 Data Management and Analysis

Data downloaded from Vitek database was transferred to a code-secured MS Excel spreadsheet. It was then cleaned continually as the study progressed.

Variables that were studied included:

Dependent: - Isolates from the respective sterile sites i.e. CSF, blood, peritoneal and pleural fluid.

- Antibiotic susceptibility patterns of these isolates.

Independent: - Age and sex of the patients.

For analysis, MS Excel spreadsheet was imported to Statistical Package for Social Sciences Programme (SPSS) version 17.0. The data was then analyzed for demographic characteristics, isolate outcomes and antibiotic susceptibility patterns.

This was carried out in two stages:

i) Univariate analysis

The Univariate analysis involved summarization and graphical presentation of both categorical and continuous variables. Categorical variables were summarized using frequency distributions and presented using bar charts and frequency distribution tables.

ii) Bivariate analysis

Bivariate analysis was used to investigate any association between variables. The χ^2 test was used to test association between 2 variables if they were categorical and satisfied all the conditions. If some χ^2 conditions were not be met, Fisher's exact test was used instead.

The raw data was then stored in a secured MS-Excel spreadsheet in a safe locker in UNITID till after publication.

STUDY LIMITATIONS

1. Missing data of some of the computerized records of relevant information such as the wards and sample type necessitated going back to the archives for confirmation.
2. Analysis of synovial fluid could not be carried out as there was no growth obtained from any of the samples.

CHAPTER FOUR.

4.1 RESULTS

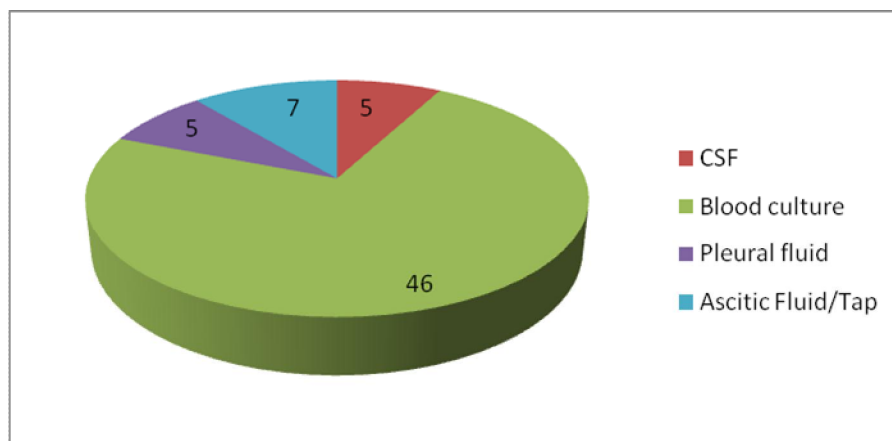
During the twelve month study period, majority of the general population from whom samples were obtained were female at 63.5% in comparison to male who were 36.5%.

Table 1: Demographic Characteristics

Characteristics	N	%
Gender		
Male	23	36.5
Female	40	63.5
	63	100

Sterile site samples obtained were mainly from blood culture at 73% (46/63), followed by ascitic fluid 11.1% (7/63) then pleural fluid and CSF both at 7.9% (5/63) each. No isolate was obtained from synovial fluid.

Figure 1: Sample source distribution



According to ward location, Newborn unit seemed to dominate with 54% of isolates arising from there, followed by the general adult wards (23.8%), followed by paediatrics ward (15.9%) with a small percentage from renal unit (3.2%) , radiology and Intensive care unit (1.6% each).

Figure 2: Ward distribution

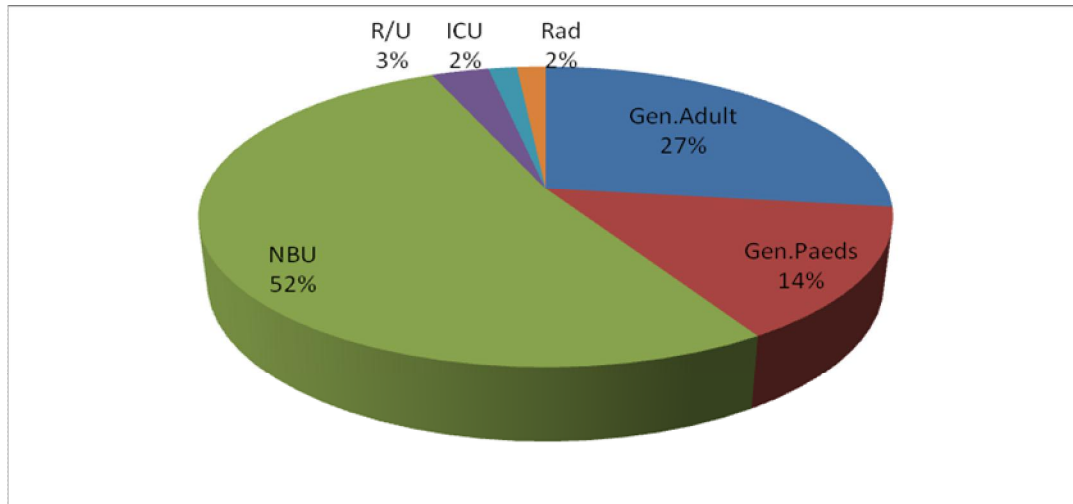


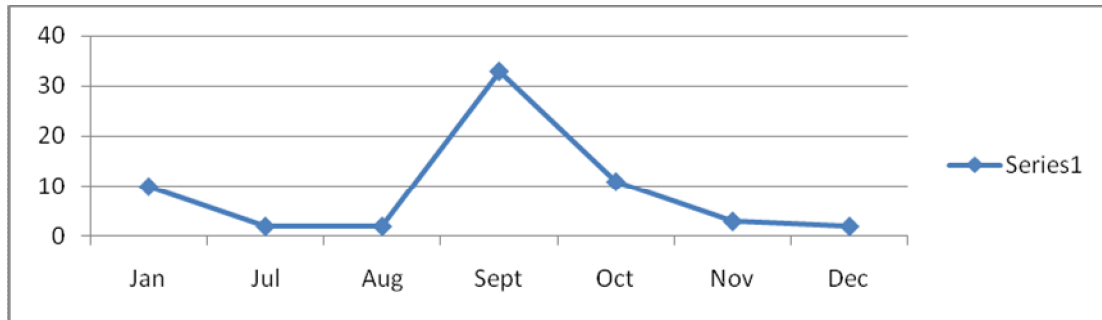
Table 2: Isolation rate

The isolation rate for each of the sterile samples collected over the year 2013 were as follows:

Sample	Total collected	Total cultured	Isolation rate (%)
CSF	2,088	5	0.24
Blood	2,852	46	1.6
Ascitic fluid	263	7	2.66
Pleural fluid	262	5	1.91
Synovial fluid	49	0	0

The month with the highest number of isolates was September with 33 isolates whereas from February to June no isolates were identified, and the lowest being 2 in July, August and December.

Fig. 3: Isolation by Months



Organisms isolated

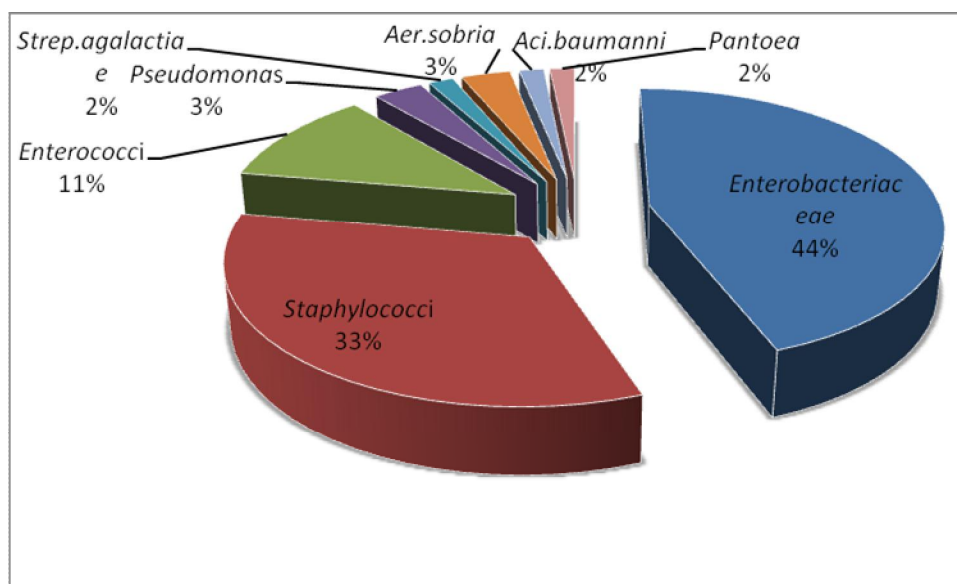
The following organisms were identified:

Table 3

Organisms	N	%
1. <i>Staphylococci</i>	21	33.3
<i>Staph.aureus</i>	3	4.8
<i>Other Staph</i>	18	28.6
2. <i>Enterobacteriaceae</i>	28	44.4
<i>E.coli</i>	6	9.5
<i>Klebsiella</i>	19	30
<i>E.cloacae</i>	2	3.2
<i>S.marcescens</i>	1	1.6
3. <i>Pseudomonas</i>	2	3.2

4. <i>Enterococci</i>	7	11
<i>E.faecalis</i>	4	6.4
<i>E.faecium</i>	2	3.2
<i>E.casseliflavus</i>	1	1.6
5. Others		
<i>Aci.baumannii</i>	1	1.6
<i>St.agalactiae</i>	1	1.6
<i>Aer. Sobria</i>	2	3.2
<i>Pantoea</i>	1	1.6

Fig.4: Distribution of Organisms Isolated



The following were the organisms isolated and their susceptibility patterns.

Table 5: *Staphylococci*

	Vancomycin
Sensitive	17
Resistant	4

Table 6: *Enterobacteriaceae*

	Ampicillin	PZP	Ceftazidime	Cefuroxime	Cefoxitin	Cefepime	Amikacin	Meropenem
Sensitive	2	22	14	1	18	18	26	26
Resistant	23	5	14	26	9	9	1	2

Table 7: *Pseudomonas*

	Ampicillin	PZP	Ceftazidime	Cefuroxime	Cefoxitin	Cefepime	Amikacin	Meropenem
Sensitive	0	0	2	2	2	1	2	1
Resistant	2	2	0	0	0	1	0	1

Table 8: *Enterococci*

	Ampicillin	Vancomycin
Sensitive	1	5
Resistant	0	2

Table 9: *Aci.baumannii*

	Ampicillin	PZP	Ceftazidime	Cefuroxime	Cefoxitin
Sensitive	0	0	0	0	0
Resistant	1	1	1	1	1

Table 10: *Strep. Agalactiae*

	Ampicillin	Vancomycin
Sensitive	1	1
Resistant	0	0

Table 11: *Aer.sobria*

	Ampicillin	PZP	Ceftazidime	Cefuroxime	Cefoxitin	Cefepime	Amikacin	Meronem
Sensitive	0	1	0	0	0	2	2	1
Resistant	2	1	2	2	2	0	0	1

Table 12: *Pantoea*

	PZP	Ceftazidime	Cefepime	Meronem
Sensitive	0	0	1	1
Resistant	1	1	0	0

Table 12: Association between Gender and Organisms isolated.

The following P-values were found showing a difference but were not statistically significant and therefore showing no association between gender and the organisms isolated.

Characteristics	Male (n=23)	Female(n=40)	
Organism			
<i>Enterobacteriaceae</i>	9(39.1%)	19(47.5%)	0.571
<i>Enterococci</i>	2(8.7%)	5(12.5%)	
<i>Pseudomonas</i>	1(4.3%)	1(2.5%)	
<i>Strep.agalactiae</i>	0(0%)	1(2.5%)	
<i>Aer.sobria</i>	0(0%)	2(5.0%)	
<i>Aci.baumannii</i>	0(0%)	1(2.5%)	
<i>Pantoea</i>	1(4.3%)	0(0%)	
Staph	10(43.5%)	11(27.5%)	

Table 12: Association between Gender and Antibiotic Susceptibility Patterns.

The following P-values were found showing a difference but were not statistically significant and therefore showing no association between gender and antibiotic susceptibility patterns.

	Males(n=23)	Female(n=40)	
Ampicillin			
Sensitive	1(10.0%)	4(16.0%)	0.647
Resistant	9(90.0%)	21(84.0%)	
Amikacin			
Sensitive	10(100%)	20(95.2%)	0.483
Resistant	0(0%)	1(4.8%)	
Ceftazidime			
Sensitive	5(45.5%)	11(47.8%)	0.897
Resistant	6(54.5%)	12(52.2%)	
Cefuroxime			
Sensitive	0(0%)	1(4.5%)	0.493
Resistant	10(100%)	21(95.5%)	
Cefepime			
Sensitive	7(63.6%)	15(68.2%)	0.794
Resistant	4(36.4%)		

Cefoxitin			
Sensitive	5(50%)	14(63.6%)	0.467
Resistant	5(50%)	8(36.4%)	
Meropenem			
Sensitive	9(81.8%)	21(95.5%)	0.199
Resistant	2(18.2%)	1(4.5%)	
PiperacillinTazobactam			
Sensitive	9(81.8%)	17(77.3%)	0.763
Resistant	2(18.2%)	5(22.7%)	
Vancomycin			
Sensitive	9(75%)	14(82.4%)	0.63
Resistant	3(25%)	3(17.6%)	

4.2 DISCUSSION

The aim of this study was to identify bacterial pathogens identified from samples collected in sterile sites and their antibiotic susceptibility patterns.

4.2.1 CSF

In the present study, an isolate each of *E.coli* and *Staphylococci* were isolated from both adults and children. This was partly in keeping with previous studies by Chandramukhi (1989), Chinchankar (2002), and Sonavane(2008) though the main organisms they found ie *N.meningitidis*, *S.pneumoniae* and *H.influenzae* in infants and children were not isolated. This could be because of the increased uptake of childhood vaccines. No isolates were obtained from the neonatal group. *P.aeruginosa* was isolated from the paediatric age group with the sample having been obtained from an infected ventriculoperitoneal (VP) shunt in the surgical ward. These findings can also be attributed to usage of antibiotics prior to collection of CSF samples in peripheral clinics before referral.

P.aeruginosa is one of the most common nosocomial pathogens causing iatrogenic meningitis (Talbot) and has greater ability to develop resistance to virtually any antibiotic it's exposed to because of multiple resistance mechanisms that can be present concurrently within the pathogen (Livermore DM, 2001). In this study an incidence of 16% was found compared to that of 66% in two different studies by Navageni (2011) and Jones (1997). Although lower, this incidence is still high as this organism is mainly found in critical patients whose immunity is low.

4.2.2 Blood cultures

Blood cultures had the highest number of samples collected over the year 2013 and also yielded the highest number of isolated pathogens in comparison to other samples collected from sterile sites. A majority of the samples were from the newborn unit.

In this study, the majority of isolates were Klebsiella species (37%) with 82% being from the newborn unit, followed by Coagulase negative Staphylococci (30.4%), then Enterococci (10.9%). This is in contrast to a previous study done by Deverick (2014) whose findings were *S.aureus* (28%) followed by *E.coli* (24%) then *Coagulase Negative Staphylococci* (10%). The observed emergence of *K.oxytoca* which is an opportunistic pathogen associated with nosocomial infections may be due to poor infection control measures in the newborn unit, overcrowding and also the fact that newborns themselves are susceptible to infections due to immature immune systems. In addition, lack of standard facilities with controlled air flow, entry and exit points and proper sterilization of all gowns and equipment are also contributory. The high rate of growth of CoNS may also be due to poor infection control or swabbing practices.

4.2.3 Ascitic fluid

All positive cultures of ascitic fluid were from adult patients from medical wards with none from the surgical wards. This could be attributed to misclassification of ascitic taps under pus aspirates or surgical site infections. The main organisms found in this study were *Enterobacteriaceae* (57%) and *Enterococci* (29%). 50% (2/4) of *Enterobacteriaceae* were from peritoneal dialysis fluid in the renal unit. This was in keeping with a study done by Montravers (2009) to identify microbiological profiles of intra-abdominal infections found *E.coli* to be the highest (27%), followed by Anaerobes (23%) and *Streptococci*(12%) . On the contrary, findings by Reuken (2012) which isolated *Enterococci* as 50% of gram positive bacteria in spontaneous bacterial peritonitis and 28% in secondary peritonitis .Only one patient was found to have *S.epidermidis* which is usually peritoneal dialysis related primarily due to touch contamination (Finkelstein, 2002) but in this case we were not able to identify whether this patient had had dialysis before or not.

4.2.4 Pleural fluid

Pleural fluid isolates found were *Klebsiella* and *Staphylococcus* species, in keeping with previous studies by Foster (2007) and Schultz (2004). An interesting finding is that of *Aci. Baumannii* isolated from a child in the general paediatric ward. This is an organism that is becoming increasingly nosocomial and has been identified by Rice (2008) as an ESKAPE pathogen (*Enterobacter*, *S.aureus*, *K.Pneumonia*, *A.baumannii*, *P.aeruginosa*, *E.faecium*) whose drug of choice for treatment is carbapenems (Talbot, 2006).

4.2.5 Antibiotic Susceptibility Patterns.

Among *Staphylococci*, 81% were sensitive to vancomycin which was the only drug that was tested in VITEK 2 at KNH. Other antibiotic susceptibility tests were done using antibiotic disk inhibition method. Out of the 21 that were tested, 4 (19%) showed resistance against vancomycin and only 25% of these were *S.aureus*. These findings are contrary to a study done in neonates by Qu (2010) whereby most coagulase-negative staphylococcal isolates were resistant to penicillin G (100%), gentamicin (83.3%) and oxacillin (91.7%) and susceptible to vancomycin (100%). A different study in Benin by Sina (2013) looking at 136 isolates of *S.aureus* strains from furuncles, pyomyositis, abscesses, Buruli ulcers, and osteomyelitis, from hospital admissions and out-patients found all strains to be resistant to benzyl penicillin, while 25% were resistant to methicillin, and all showed sensitivity to vancomycin. Further local studies are necessary to establish staphylococcal susceptibility patterns to vancomycin to ascertain whether it can still be a drug of choice considering the upward trend of antimicrobial resistance.

The *Enterobacteriaceae* showed sensitivity against Piperacillin/Tazobactam, cefoxitin, cefepime, amikacin and meropenem, and resistance against ampicillin and cefuroxime. Against ceftazidime they had 50% sensitivity and 50% resistance. These findings are consistent with a local study by Kebira (2012) where all isolates of *E.coli* (100%) were susceptible to Ticarcillin,

Piperacillin/Tazobactam, Amikacin, Ofloxacin; and 80% of the isolates were susceptible to Gentamycin, Norfloxacin, and Ceftazidime.

Isolates of *K. oxytoca* were susceptible to ampicillin-sulbactam, imipenem, aminoglycosides and fluoroquinolones in a different study (Lin,1997), showing a similar pattern to the same family of antibiotic group but different molecular structure.

Pseudomonas isolates both showed sensitivity towards ceftazidime and amikacin as well. Against meropenem and cefepime 50% sensitivity and 50% resistance was found. Resistance was noted against ampicillin, piperacillin/tazobactam, cefuroxime and cefoxitin. In 2002, 14 % multidrug resistant isolates were found by Obritsch where isolates were resistant to 3 out of 4 drugs: ceftazidime, ciprofloxacin, tobramycin and imipenem. Further studies are necessary to identify drugs that can curb this trend.

Enterococci, most of which were isolated from blood, were found to be sensitive towards ampicillin but majorly towards vancomycin. On the contrary previous studies seem to contradict our findings. One study by Reuken showed 63% resistance to ampicillin and 13% to vancomycin and a different study has shown resistance of vancomycin to be on the increase with a rate of ~60% among *E. faecium* isolates (Wisplinghoff, 2004).

Findings by Landman (2002) revealed that many isolates of *Ac. baumannii* are now resistant to all aminoglycosides, cephalosporins, and fluoroquinolones, similar to findings in this study where the only isolate was resistant to all drugs tested against it: ampicillin, piperacillin/tazobactam, ceftazidime, cefuroxime and cefoxitin. There's need for further development of drugs against it.

St. agalactiae was sensitive towards both ampicillin and vancomycin, in keeping with findings from a study by Simoes (2004).

Aer. sobria was sensitive towards cefepime and amikacin. An intermediary pattern towards piperacillin/tazobactam and meropenem with 50% sensitivity and resistance was shown. Resistance against ampicillin, ceftazidime, cefuroxime and ceftiofur were noted. This was in contrast to a Taiwanese study which looked at *Aeromonas* though primarily, *A. hydrophila*. The strains were found to be susceptible to moxalactam, ceftazidime, cefepime, aztreonam, imipenem, amikacin, and fluoroquinolones, but they were more resistant to tetracycline, trimethoprim-sulfamethoxazole, some extended-spectrum cephalosporins, and aminoglycosides than strains from the United States and Australia.

Pantoea showed sensitivity towards cefepime and meropenem and resistance against ceftazidime and piperacillin/tazobactam. A Texas Children's Hospital retrospective study (January 2000 to December 2006) reviewed culture-positive *P. agglomerans* records. This organism was identified in 88 patient cultures. For the 53 children whose sterile-site cultures grew *P. agglomerans*, the isolates were uniformly susceptible to amikacin, gentamicin, meropenem and trimethoprim-sulfamethoxazole. In addition, 92.5% of isolates were susceptible to broad-spectrum cephalosporins and semisynthetic penicillins, 62.3% to extended-spectrum cephalosporins, and 47.2% to ampicillin. Our findings are similar to those of this study and the two drugs can be recommended for treatment of *Pantoea*.

4.3 CONCLUSION

Emerging ESKAPE (*Enterobacter*, *S.aureus*, *K.Pneumonia*, *A.baumannii*, *P.aeruginosa*, *E.faecium*) organisms have been isolated in this study and remain important pathogens as far as infections in sterile sites are concerned. Commonest organisms from blood were *Staphylococci* and *Klebsiella*, and from ascitic fluid were *Enterobacteriaceae*.

Among *Staphylococci*, 81% were sensitive to vancomycin which was the only drug that was tested.

The *Enterobacteriaceae* showed sensitivity against Piperacillin/Tazobactam, ceftazidime, cefepime, amikacin and meropenem, and resistance against ampicillin. These drugs can still be used with confidence in patients with suspected enterobacteriaceae infections.

Pseudomonas isolates both showed sensitivity towards ceftazidime and amikacin and multidrug resistance against ampicillin, piperacillin/tazobactam, cefuroxime and ceftazidime. Further studies are necessary to identify drugs that can curb this trend.

Further studies need to be done especially concerning novel antibiotics to establish the best drugs that can be used to tackle these bacteria.

Recommendations:

1. Surveillance especially for the emerging pathogens needs to be carried out judiciously to help develop rational antimicrobial guidelines alongside continuous medical education .
2. Rational use of antibiotics is advised to help curb this trend of increasing antibiotic resistance.
3. Infection prevention and control interventions in the hospital such as promotion of proper personal hygiene and controlled environment eg. in newborn units is necessary to help control nosocomial infections.
4. Subsidizing the cost of some of the very expensive antibiotics like vancomycin will help with the treatment especially of the emerging organisms.

CHAPTER 5

5.1 TIMELINE

	2014							
	April	May	June	July	Aug	Sept	Oct	Nov
Proposal submission and Ethical approval	X	X	X					
Data Abstraction				X	X			
Data analysis and Report writing						X	X	
Project Defense								X

5.2 BUDGET

ITEM	COST (Kshs)
1. Ethics fees	2,000
2. Statistician	30,000
3. Operating expenses: stationery, Printing, photocopying etc	35,000
4. Miscellaneous	10,000
5. Contingency	7,700
TOTAL	84,700

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5.4 APPENDIX

5.4.1 Data Collection Form

**ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF BACTERIA ISOLATED FROM
STERILE SITES: CEREBRAL SPINAL FLUID, BLOOD, PERITONEAL
FLUID, PLEURAL FLUID AND SYNOVIAL FLUID AT KENYATTA NATIONAL
HOSPITAL.**

Sample No:

Socio-Demographic characteristics

Age of Patient : í í í í Yrs

Sex Male
 Female

Sample: CSF Blood Culture Peritoneal fluid
 Pleural Fluid Synovial Fluid

Month in which organisms were isolated.

January	<input type="checkbox"/>	February	<input type="checkbox"/>
March	<input type="checkbox"/>	April	<input type="checkbox"/>
May	<input type="checkbox"/>	June	<input type="checkbox"/>
July	<input type="checkbox"/>	August	<input type="checkbox"/>
September	<input type="checkbox"/>	October	<input type="checkbox"/>
November	<input type="checkbox"/>	December	<input type="checkbox"/>

Area from which specimen was obtained

ICU	<input type="checkbox"/>	Paeds	<input type="checkbox"/>	Gen Ward	<input type="checkbox"/>
Renal Unit	<input type="checkbox"/>	Burns	<input type="checkbox"/>	OPD	<input type="checkbox"/>
	<input type="checkbox"/>				

Others

Organisms Isolated: í í í í í í í í í í í í í í í ..

Antibiotic Susceptibility Patterns

Drug	Sensitive	Resistant
Ampicillin		
Amoxicillin/Clavulanic Acid		
Amoxicillin		
Amikacin		
Beta-Lactamase		
Chloramphenicol		
Ceftazidime		
Cefalotin		
Ciprofloxacin		
Clindamycin		
Cefpodoxime		
Ceftriaxone		
Cefotaxime		
Cefuroxime		
Erythromycin		
Fusidic Acid		
Cefepime		

Fosfomicin		
Cefoxitin		
Nitrofurantoin		
Gentamicin		
Imipenem		
Levofloxacin		
Linezolid		
Meropenem		
Mupirocin		
Moxifloxacin		
Norfloxacin		
Ofloxacin		
Oxacillin		
Benzylpenicillin		
Piperacillin		
Quinupristin/Dalfopristin		
Rifampicin		
Ampicillin/Sulbactam		
Trimethoprim/Sulfamethoxazole		
Tetracycline		
Teicoplanin		
Telithromycin		
Tobramycin		

Piperacillin/Tazobactam		
Vancomycin		

5.4.2 Laboratory Procedures

I. SAMPLE COLLECTION

a)CSF :

Before aspirating the specimen the skin is disinfected then the sample is collected in a sterile screw-cap tube. Rapid testing such as Gram stain and cryptococcal antigen should be considered. For direct examination, best sensitivity for Gram staining is achieved after centrifugation.

It is transported immediately to the lab and can be stored for 6 hours at room temperature before processing. Primary plating media is Blood Agar (BA) and Chocolate Blood Agar (CBA).

b)Blood culture:

The venipuncture site is disinfected with 70% alcohol and disinfectant such as betadine. Blood should be drawn at the time of the febrile episode and if possible before antimicrobial treatment has started. Two sets are drawn from right and left arms, not more than three sets should be drawn in a 24hour period. For adults draw > 20ml/set and for children 1-20mls/set depending on patient's weight and blood culture bottles' manufacturer's instructions. It should be transported to the laboratory within 2hours of collection, and should be incubated at 37°c.

c)Effusions:

The skin is disinfected before aspirating the specimen using a needle. The sample is collected in a universal sterile, screw-cap tube or anaerobic transporter and should be transported immediately to the laboratory, with a maximum of 6 hours for ascitic fluid. Plating should be done as soon as received using both aerobic and anaerobic blood culture bottle set for body fluids. Gram staining is then done. One may need to concentrate by centrifugation or filtration then stain and culture the sediment.

II. SAMPLE PROCESSING

a) CSF

Day 1: Report appearance by describing whether it is clear, slightly turbid, cloudy or purulent and whether it contains blood or clots.

Testing c.s.f.:

- Purulent or cloudy c.s.f.: Suspect pyogenic bacterial meningitis and perform a Gram smear then report on the number of pus cells and on bacteria.

- Slightly turbid or clear c.s.f.: Perform cell count and note whether there are pus cells or lymphocytes. If pus cells then do a gram smear and then culture. If lymphocytes measure protein and glucose and do a Ziehl-Nielsen stain for Acid Fast Bacilli (AFB) and Indian Ink for encapsulated yeasts.

b) Blood culture

Using an aseptic technique, dispense 10-12 ml blood into Columbia agar diphasic medium and mix. Incubate up to 7 days (4 weeks when brucellosis is suspected). When growth occurs it is subcultured on BA and CBA for identification. When anaerobic infection is suspected, dispense 5 ml blood into thioglycollate broth and mix. Incubate up to 14 days.

The blood broth is allowed to run over the slope by tipping the bottle at regular intervals. Microbial activity can be seen by growth on the slope (beginning at the broth-agar interface).

3.Effusions:

Day 1 - Describe colour and whether the specimen is clear, cloudy, or purulent; blood stained or contains clots (non-citrated sample).

If pus: Do a gram smear and microscopically examine for pus cells and bacteria, and Ziehl-Nielsen smear when tuberculosis or *M. ulcerans* disease is suspected.

- Potassium Hydroxide (KOH) preparation: when a fungal or actinomycete infection is suspected.

-Giemsa or Wayson's smear: when bubonic plague is suspected.

-Polychrome methylene blue: when cutaneous anthrax is suspected.

-Dark-field microscopy: To detect treponemes when yaws or pinta is suspected.

If blood stained: proceed to culture.

Examine by estimating cell numbers and report % of cells that are neutrophils or lymphocytes.

For protein: report total protein in g/l.

- Transudate: Clear unclotted fluid with few cells and protein below 30 g/l: No need to test further.

- Exudate: When cloudy fluid with more than a few cells and protein over 30 g/l do culture and examine microscopically.

- Gram smear - microscopically look for pus cells and Ziehl-Nielsen look for AFB.

III SAMPLE CULTURE AND IDENTIFICATION

a)CSF

Day 2 and onwards, culture CSF.

On BA and CBA. Incubate in CO₂

ó If neonate: also MacConkey agar. Incubate aerobically.

On BA and CBA look particularly for:

- . *Neisseria meningitidis* (growing on chocolate agar and blood agar, oxidase positive)
- . *Streptococcus pneumoniae* (sensitive to optochin)
- . *Haemophilus influenzae* (growing only on chocolate agar)
- . *Cryptococcus neoformans* (Gram stain the colonies)

On MacConkey agar culture look especially for bacteria that cause neonatal meningitis.

b) Blood culture

Day 2 and onwards examine and report cultures. After overnight incubation examine diphasic culture. Subculture (even when no growth is seen at end of 7 days for aerobic incubation or 14 days for anaerobic incubation) on BA and incubate aerobically or anaerobically. For CBA incubate in CO₂.

Examine thioglycollate culture. Subculture and examine microscopically. Incubate subculture anaerobically.

Note: When there is no growth, wash slope of diphasic culture. Reincubate cultures. Subculture as indicated.

Examine subcultures for likely pathogens.

c) Effusions

Culture specimen on BA and incubate aerobically. For CBA incubate in CO₂ and for MacConkey which is rarely used incubate aerobically.

Culture for *M. tuberculosis* requires facilities of a Tuberculosis Reference Lab on Lowenstein Jensen Medium and examined for growth after 6 weeks.

Examine and report cultures: BA, CBA, MacConkey.

Look particularly for: *S. aureus*, *S. pyogenes*, *S. pneumoniae*, *H. influenzae*, *Neisseria* species, Enterobacteria, *P. aeruginosa*.

IV ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST)

This is done for all the above samples with growth after subculturing on Muller-Hinton Agar. Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial susceptibility. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on the plate of susceptibility testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related (among other factors) to the susceptibility of the organism. Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc.

In VITEK 2, the AST is done automatically with appropriate cassettes and results produced automatically and where in doubt, conventional methods used to confirm identification and AST results.

5.4.3 Vitek 2

The Vitek 2 System (bioMérieux) is highly automated and uses very compact plastic reagent cards (credit card size) that contain microliter quantities of antibiotics and test media in a 64-well format. Each card has a pre-inserted transfer tube used for inoculation. Cards have bar codes that contain information on product type and a unique identifier that can be linked to the sample either before or after loading the card onto the system.

There are currently four reagent cards available for the identification of different organism classes :

1. GN - Gram-negative fermenting and non-fermenting bacilli
2. GP - Gram-positive cocci and non-spore-forming bacilli
3. YST - yeasts and yeast-like organisms
4. BCL - Gram-positive spore-forming bacilli

Suspension Preparation

A sterile swab or applicator stick is used to transfer a sufficient number of colonies of a pure culture and to suspend the microorganism in 3.0 mL of sterile saline in a 12 x 75 mm clear plastic (polystyrene) test tube.

Inoculation

Identification cards are inoculated with microorganism suspensions using an integrated vacuum apparatus. A test tube containing the microorganism suspension is placed into a special rack (cassette) and the identification card is placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The filled cassette is transported automatically into a vacuum chamber station. After the vacuum is applied and air is re-introduced into the station, the organism suspension is forced through the transfer tube into micro-channels that fill all the test wells.

Card Sealing and Incubation

Inoculated cards are passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. Each card is removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next read time. Data are collected at 15-minute intervals during the entire incubation period.

Optical System

A transmittance optical system allows interpretation of test reactions using different wavelengths in the visible spectrum. During incubation, each test reaction is read every 15 minutes to measure either turbidity or colored products of substrate metabolism. In addition, a special algorithm is used to eliminate false readings due to small bubbles that may be present.

Database Development

The databases of the VITEK 2 identification products are constructed with large strain sets of well-characterized microorganisms tested under various culture conditions. These strains are derived from a variety of clinical and industrial sources as well as from public (e.g., ATCC) and university culture collections.

Analytical Techniques

Test data from an unknown organism are compared to the respective database to determine a quantitative value for proximity to each of the database taxa. Each of the composite values is compared to the others to determine if the data are sufficiently unique or close to one or more of the other database taxa. If a unique identification pattern is not recognized, a list of possible organisms is given, or the strain is determined to be outside the scope of the database.

(Microbial Identification Using The Biomérieux Vitek[®] 2 System , David H. Pincus , bioMérieux, Inc. USA)